

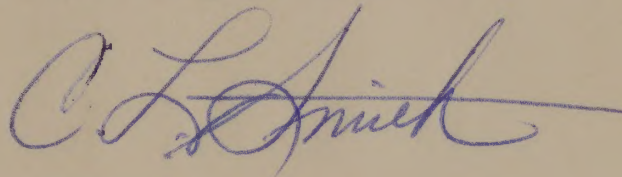
UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
ENTOMOLOGY RESEARCH BRANCH

FEB 4 1957

P. O. Box 3391
Orlando, Florida
January 30, 1957

To: R. C. Bushland, Box 232, Kerrville, Texas
From: C. L. Smith
Subject: Report--Clewiston Field Test

Enclosed are two copies of a report covering the Clewiston field test. Three copies of the report are being forwarded to Dr. Lindquist's office.



(In duplicate)
cc: (3) A. W. Lindquist

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
MEMORANDUM FOR THE DIRECTOR

F. A. Box 3531
Tulsa, Florida
January 20, 1957

R. C. Bushland, Box 233, Perryville, Texas

C. L. Smith

Report--Gleanings from the

Enclosed are two copies of a report covering the Gleanings from the
field. Three copies of the report are being forwarded to
Dr. Bushland's office.

(In duplicate)
cc: (3) A. W. Lindquist

January 30, 1957

Field Test Comparing Release of Sterile Screw-worm
Flies in 1-mile Swaths and 3-mile Swaths

Although the possibility of eradicating the screw-worm fly through the release of sterile male flies was clearly demonstrated on Curacao in 1954, several questions remain unanswered regarding the most effective and efficient means of distribution on a large scale as proposed for the Florida eradication program. Among these unanswered questions are swath width, time interval between releases, feeding before release and holding the emerged flies for 2 or 3 days prior to release (until ready to mate). In the test reported here releases were made to determine if releasing the sterile flies in 3-mile swaths would be as effective as releasing them in 1-mile swaths.

Selection of Test Area

Since the test was to be conducted in October and November, Southern Florida was selected as the test site to insure an adequate screw-worm population for sampling. Preliminary selection of plots for the test was limited to areas accessible by auto. However, four 400 square mile plots were available southwest of Lake Okeechobee (see map). Liver-baited fly trap collections indicated that 3 of the 4 areas contained sufficiently uniform screw-worm populations to be used in the test. These fly trapping data are tabulated in table 1. Highways in areas 1, 3, and 4 allowed the location of traps in a straight line running north and south through the center of the areas with the 5 traps being spaced at 4-mile intervals in

in each area. However, in area 2 it was necessary to place traps east and west of an imaginary center line because of limited access by auto.

Goat-pen Locations

Since screw-worms were most abundant in areas 1, 2, and 3 these 3 plots were selected for the test and goat pens were established on October 8. Three pens were established in each area, 1 pen being in the center with additional pens approximately 2 miles north and 2 miles south of the central pen. Six to 8 goats, depending on size, were placed in each pen. Initially 2 goats were wounded and infested with screw-worms in each pen with an additional goat being infested every 3 to 4 days. From 4 to 5 attractive wounds were available as oviposition sites after 2 1/2 weeks and throughout the remainder of the experiment.

Concurrently with the establishment of goat pens the liver-baited fly traps were reduced to 2 each in the 3 areas selected for the test. Fly-trapping data during this period are tabulated in table 2.

During the first 2 weeks egg-mass collections (data are tabulated in table 3) in area 1 totaled 150, 158 in area 2, and 111 in area 3. Consequently areas 1 and 2 were selected for release with area 3 being observed as the untreated check to indicate population trends in the absence of sterile males.

Rearing

Five groups of screw-worms were reared at five- and six-day intervals for the Clewiston field releases. All flies for this test were reared in

6 large trays 9.5 x 4.5 feet and 1.5 inches deep. Each tray formed the top of a box-like enclosure and was heated from beneath with light bulbs.

There were 2 cultures of larvae started on successive days for each release group. This was necessary in order to spread out the pupation periods for sterilization at the proper time. Because of the five-day interval between cultures, newly hatched larvae were started in small trays and transferred to the large trays after 24 hours. The larvae were then given new media once daily in sufficient quantity that feeding at night was unnecessary.

Although much of the rearing was manhandled considerable labor was saved by utilization of the large trays and media mixing equipment. The technique of egging in jars instead of vials required only about one-tenth as much time. The disposal of spent media, sifting and handling of pupae, and colony maintenance required about twice the labor as growing the larvae.

Some data on the five larval rearings are shown in the following tabulation:

<u>Rearing</u>	<u>Number of pupae</u>	<u>Number of pupae per cc.</u>	<u>Pounds of heart per 1,000 larvae.</u>	<u>Percent emergence after irradiation</u>
1st	720,000	9.0	1.3	92
2nd	588,000	8.4	1.5	90
3rd	506,000	9.2	No record	91
4th	646,000	8.3	1.7	87
5th	739,500	8.5	1.5	90

6 large trays 9.5 x 4.5 feet and 1.5 inches deep. Each tray formed the top of a box-like enclosure and was heated from beneath with light bulbs. There were 2 cultures of larvae started on successive days for each release group. This was necessary in order to spread out the pupation periods for sterilization at the proper time. Because of the five-day interval between cultures, newly hatched larvae were started in small trays and transferred to the large trays after 24 hours. The larvae were then given new media once daily in sufficient quantity that feeding at night was unnecessary.

Although much of the rearing was handled by considerable labor was saved by utilization of the large trays and media mixing equipment. The technique of rearing in jars instead of vials required only about one-tenth as much time. The disposal of spent media, sitting and handling of pupae, and colony maintenance required about twice the labor as growing the larvae. Some data on the five larval rearings are shown in the following

tabulation:

Rearing	Number of pupae	Number of pupae per cc.	Pounds of heart per 1,000 larvae.	Percent emergence after irradiation
1st	730,000	9.0	1.3	92
2nd	582,000	8.4	1.5	90
3rd	506,000	9.2	No record	91
4th	646,000	8.3	1.7	87
5th	732,500	8.5	1.5	90

Sterilization and preparation for shipment

An estimated total of 3,199,500 pupae were reared and from these 2,320,000 were irradiated for release. Pupae were held at 80°F. and sterilized with 7,500 r of irradiation at 5 days of age, plus or minus 12 hours. It required about 54 hours of continuous irradiation time for each release. In order to have all the flies emerge near the same time, pupae irradiated during the first 24 hours were held at 60°F. for the following 24 hours.

The preparation of 4,114 containers for each release, along with the sterilization, required more man power than rearing the flies.

Following irradiation a sufficient number of pupae were measured into each release carton to provide approximately 100 emerged flies. This number was determined after preliminary examination of pupae for probable emergence. Into each carton was placed a ball of excelsior to absorb moisture and to provide additional resting area for the flies.

Release cartons used were waxed Klik-lok 4" x 5" x 1 1/2"^{boxes}/as used for packaging frozen foods. These boxes were designed for machine folding and involved considerable time for hand-folding for our use. However, cartons designed for hand-folding were not sufficiently "fly-proof" to be utilized as previous experience indicated newly emerged screw-worm flies were very adept at escaping through openings as small as 1/16". Estimated numbers of flies escaping from the release cartons ranged from 0.5% to 1% as counted on windows and screens in the laboratory. Extensive tests involving

weighting of cartons and holding in darkness gave variable results indicating that care used in folding the cartons was the primary factor in the escape of flies. However, weighting individual stacks of cartons with 2" x 4" x 5" wooden blocks was considered helpful in reducing the number of escaped flies.

Feeding Flies in Release Cartons

Since flies were to be released on the 3rd day following emergence it was necessary to provide food for them. Prior to release 8 feeding methods were tested placing sufficient pupae in small cages 12" x 6" x 6" to provide approximately 100 flies in each cage. Four cages of flies were used for each method. At the end of 3 days food and water were removed to obtain an indication of the food reserve built up by flies feeding on the various materials.

For 3 days flies feeding on a honey-meat mixture, honey-meat plus water, honey alone, 50% honey, honey alone plus water, and on sugar lumps plus water lived very well with mortality ranging from 0.25% to 1.7%. Two days following the removal of the food, flies that had fed on 50% honey alone and on sugar lumps alone plus water showed the lowest mortality, 38.2%. However, since flies fed on honey alone also lived well it was decided arbitrarily to use cotton saturated with 75% honey since this method provided the simplest method of feeding with least danger of spillage in the release carton. Data from this test are contained in table 4.

Shipment of Flies to Field Test Laboratory

To avoid the difficulties involved in depending on commercial transportation, the release cartons, packed in large cartons, were loaded on a pick-up truck, covered with a tarpaulin and hauled from the Orlando laboratory to the field. Upon receipt at the field laboratory the cartons were unpacked and placed on tables. This was necessary since considerable heat is generated when emergence of the flies begins. In fact on the emergence tables it was necessary to provide at least a 1-inch spacing between the stacks of flies to prevent overheating during emergence and adult activity.

Since mating activity of the males does not reach a peak until the third day of adult life at 80°F. the flies were not released until the third day after emergence, on the theory that natural predation would be decreased.

Dispersal of Flies

Flies were dispersed over the release areas with a 65 hp. 2-place Luscombe 8A. The pilot skillfully provided the 12 cu. ft. of space required for 684 cartons dropped each flight, by replacing the canvas baggage compartment with a larger compartment extending toward the rear of the fuselage. A removable back rest was provided to facilitate loading of the plane. Weight of the 684 cartons was 50 pounds, within the baggage limit of 55 pounds.

Several release tubes were devised by the pilot and attached to the right-hand door of the plane. However, it was found that dispersal of the cartons could be accomplished very well by merely flinging the cartons through an open window.

Prior to release the cartons were pierced with a sharp-pointed punch tapered to 5/8" at the base. The hole was made on a narrow edge of the carton to prevent submergence of the carton should it fall into a watered area.

In area 1 and the northern half of area 2 yellow flag markers and existing landmarks facilitated determination of flight lanes. However, inaccessibility and lack of landmarks in the southern half of area 2 limited navigation to compass readings and accurate timing with a stop watch. The sweep-hand of the stop watch was also used to determine dropping intervals of the cartons which varied from 5 to 10 seconds in area 1, depending upon ground speed as affected by wind direction and velocity. In area 2 where the dropping interval was one-third that of area 1 it was necessary to reduce ground speed, when accelerated by a strong tail wind, to limit the dropping interval to a minimum of 2.4 seconds or 25 cartons per minute, since it was humanly impossible to disperse the cartons any faster than this rate.

To limit variation in release conditions the experimental design called for releases in both areas on the same day. Since total flying time for the 2 areas averaged 9 hours for each release, it was possible to treat both areas with 1 plane with the exception of from 2 to 4 lanes in area 1 which were usually flown early the following morning. However, variability in weather conditions from day to day during release appeared no greater than the variation between the areas on the same day.

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To limit variation in release conditions the experimental design called for releases in both areas on the same day. Since total flying time for the 2 areas averaged 9 hours for each release, it was possible to treat both areas with 1 plane with the exception of from 2 to 4 lanes in area 1 which were usually flown early the following morning. However, variability in weather conditions from day to day during release appeared no greater than the variation between the areas on the same day.

Five releases were scheduled at 5-day intervals at the rate of 285 sterile male screw-worms per sq. mi. (equals 400 per sq. mi. per week). Actual release dates varied somewhat; they are as follows: 10/23/56, 10/28/56, 11/4/56, 11/8/56, 11/13/56.

To minimize variation in the flies used in one area compared to the other, cartons of flies held in cold and not subjected to cold were distributed equally on 4 emergence tables in a lengthwise fashion. As each plane load was re-boxed for transfer to the plane an equal cross-section from each table was taken to provide a uniform group of flies for each flight.

Flight lanes were established so that the goat pens were midway between the two center flight lanes of each treated area.

Results and Discussion

Total egg mass counts for the two treated areas were quite uniform throughout the experiment indicating a fortunate selection of treated areas. However, the untreated check area which totaled only 22 egg masses from the 3 pens during the initial week eventually reached a weekly high of 257 masses. Numerous natural infestations in hogs roaming near pen seven undoubtedly contributed to this high population density.

Egg mass totals, fertile and sterile, are combined below for the period beginning one week after the first release through the second week following the final release i.e. October 30, 1956 through November 26, 1956.

Total egg masses 10/30 - 11/26/56

1-Mile swath				3-mile swath				Untreated check			
Pen	F.	S.	:Percent: sterile	Pen	F.	S.	:Percent: sterile	Pen	F.	S.	:Percent: sterile
1	36	3	7.7	4	101	14	12.2	7	519	1	--
2	81	15	15.6	5	55	21	27.6	8	203	2	--
3	<u>57</u>	<u>19</u>	<u>25.0</u>	6	<u>43</u>	<u>6</u>	<u>12.2</u>	9	<u>103</u>	<u>0</u>	<u>--</u>
Totals	174	37	17.5		199	41	17.1		825	3	--

Sterility in the 1-mile area was 17.5% compared to 17.1% in the 3-mile area indicating no advantage of the 1-mile swath over the 3-mile swath in this test.

Table 1.--Screw-worm fly trap collections (liver-baited) Clewiston, Fla. 1956, prior to selection of test site for releasing sterile screw-worms in 1-mile and 3-mile swaths.

		: 9/14-17 :		: 9/17-20 :		: 9/20-24 :		: 9/24-27 :		: 9/27-10/1 :		: 10/1-8 :			
Trap:		:cc's. :		:cc's.:		:cc's. :		:cc's. :		:cc's.:		:cc's.:		Total	
No.:		SW's.:flies		:SW's.:flies		:SW's.:flies		:SW's.:flies		:SW's.:flies		:SW's.:flies		:SW's.	
Area 1															
1	8	825	3	235	5	900	20	900	3	700	4	700	43		
2	36	190	6	65	16	300	11	160	9	335	6	165	84		
3	3	50	1	15	4	65	7	80	10	110	8	25	33		
4	7	330	2	20	5	110	3	200	0	320	0	140	17		
5	35	1,500	8	50	15	190	28	400	13	285	0	100	99		
Total	89		20		45		69		35		18		276		
Area 2															
6	0	5	0	5	1	10	0	80	0	10	0	40	1		
7	0	15	0	5	0	15	2	50	0	100	0	0	2		
8	2	90	0	5	2	50	3	150	13	185	0	20	20		
9	9	110	0	10	28	90	19	50	14	100	1	35	71		
10	0	10	1	50	7	30	73	180	17	175	3	125	101		
Total	11		1		38		97		44		4		195		
Area 3															
11	0	45	0	35	0	20	1	20	0	150	0	60	1		
12	5	50	1	35	0	50	14	230	7	750	0	0	27		
13	9	250	4	100	8	100	35	800	21	1,500	4	450	81		
14	0	50	0	5	22	1,250	1	15	0	80	0	200	23		
15	0	75	0	5	1	10	3	100	4	395	4	900	12		
Total	14		5		31		54		32		8		144		
Area 4															
16	1	2,800	4	650	2	1,650	3	1,200	1	950	1	800	12		
17	0	615	0	125	0	700	1	370	3	600	0	220	4		
18	0	310	5	510	3	500	8	400	0	1,000	0	350	16		
19	0	25	0	10	0	40	1	120	1	175	0	175	2		
20	0	25	0	5	0	20	1	15	1	10	0	0	2		
Total	1		9		5		14		6		1		36		

Table 1.--Sewer-worm fly trap collections (liver-baited) (Newston, Wis., 1936).
Prior to selection of test site for releasing sterile sewer-worms in
1-mile and 3-mile areas.

Area 1											
1	8	825	3	225	8	200	20	800	3	700	4
2	28	180	8	65	16	300	11	160	9	235	8
3	3	50	1	15	4	65	7	20	10	110	8
4	7	230	2	20	5	110	3	300	0	230	0
5	25	1,500	8	20	15	190	28	400	12	235	0
Total	59		20	45	45	69	25	25	18		278
Area 2											
6	0	5	0	5	1	10	0	80	0	10	0
7	0	15	0	5	0	15	3	20	0	100	0
8	2	30	0	5	2	50	3	120	13	185	0
9	3	110	0	10	28	90	19	20	14	100	1
10	0	10	1	20	7	30	73	180	17	175	3
Total	11		1	28	38	97	44	44	4		198
Area 3											
11	0	45	0	25	0	20	1	20	0	150	0
12	2	50	1	25	0	80	14	230	7	750	0
13	3	230	4	100	8	100	28	800	21	1,500	4
14	0	50	0	25	1	1,250	1	15	0	60	0
15	0	75	0	2	1	10	3	100	4	335	4
Total	14		5	21	31	64	35	35	8		144
Area 4											
16	1	2,800	4	850	2	1,650	3	1,200	1	950	1
17	0	615	0	155	0	700	1	270	2	600	0
18	0	210	2	610	3	800	8	400	0	1,000	0
19	0	25	0	10	0	40	1	120	1	175	0
20	0	25	0	5	0	20	1	15	1	10	0
Total	1		6	2	5	14	6	6	1		38

Table 2.--Screw-worm fly trap collections (liver baited) Clewiston, Fla. 1956, in test areas selected for a comparison of releasing sterile screw-worm flies in 1-mile and 3-mile swaths.

		: 10/9-15 ^a :		10/16-22 :		10/23-29 :		10/30-11/6 :		11/7-9 :		11/10-15 :		11/16-19 :		11/20-26 :		11/27-12/3 :		12/4-10 :		12/11-17 :	
		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :		: cc's. :	
Trap No.:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:	SW's:flies:
Area 1																							
2	75	350	16	740	26	850	74	800	40	275	36	1,300	86	3,500	53	1,700	15	215	70	590	18	400	
4	<u>2</u>	155	<u>4</u>	320	<u>20</u>	1,250	<u>31</u>	1,150	<u>7</u>	500	<u>21</u>	525	<u>23</u>	900	<u>11</u>	425	<u>6</u>	65	<u>20</u>	850	<u>4</u>	1,675	
Totals	77		20		46		105		47		57		109		64		21		90		22		
Area 2																							
7	0	75	4	130	22	200	18	1,475	4	135	12	900	70	1,400	1	95 ^b	6	220	10	775	6	1,035	
9	<u>4</u>	210	<u>3</u>	250	<u>118</u>	685	<u>125</u>	350	<u>23</u>	110	<u>28</u>	175	<u>11</u>	415	<u>1</u>	875	<u>2</u>	250	<u>6</u>	1,460	<u>3</u>	1,150	
Totals	4		7		140		143		27		40		81		2		8		16		9		
Area 3																							
12	21	770	10	365	47	1,750	111	5,900	68	1,750	49	2,100	73	925	51	925	26	560	98	3,610	236	8,925	
14	<u>3</u>	125	<u>3</u>	55	<u>26</u>	675	<u>0</u>	85	<u>1</u>	380	<u>2</u>	50	<u>6</u>	1,900	<u>2</u>	5,725	<u>2</u>	905	<u>4</u>	2,725	<u>7</u>	6,700	
Totals	24		13		73		111		69		51		79		53		28		102		243		

a/ Trap collections were made at 3- and 4-day intervals when releasing of flies did not interfere, but are combined where possible to coincide with weekly egg-mass collections.

b/ Trap was inoperative 4 days due to destruction by force unknown.

Table 3.--Weekly egg-mass collections, fertile (F) and sterile (S), in the Clewiston, Fla. test area, 1956 where sterile screw-worm flies (both sexes) were released by plane in 1-mile and 3-mile swaths at the rate of 285 males per sq. mile approximately every 5 days^a.

Pen No.	:10/9-15:	10/16-22:	10/23-29:	10/30-11/5:	11/6-12:	11/13-19:	11/20-26:	11/27-12/3:	12/4-10:	12/11-17:								
	F.	F ^b	F.	S.	F.	S.	F.	S.	F.	S.	F.	S.	F.	S.	F.	S.	F.	S.
Area 1 - 1-mile swaths																		
1	10	34	28	0	26	1	3	0	4	0	3	2	0	0	0	0	1	0
2	37	24	13	0	31	2	20	0	24	10	6	3	2	0	12	2	8	1
3	24	21	45	0	8	1	9	1	19	6	21	11	7	2	13	1	18	1
Totals	71	79	86	0	65	4	32	1	47	16	30	16	9	2	25	3	27	2
Area 2 - 3-mile swaths																		
4	20	24	47	1	33	1	36	7	27	3	5	3	2	0	7	1	0	0
5	23	14	22	2	18	6	11	3	13	9	13	3	6	5	11	2	45	3
6	34	43	23	1	7	1	9	3	25	2	2	0	0	0	1	0	6	0
Totals	77	81	92	4	58	8	56	13	65	14	20	6	8	5	19	3	51	3
Area 3 - Untreated check																		
7	13	42	87	0	169	1	130	0	107	0	113	0	80	1	115	0	99	0
8	8	37	20	1	32	0	89	0	50	0	32	2	1	0	5	1	10	0
9	1	10	10	0	11	0	38	0	15	0	39	0	57	0	70 ^c	0	29	1
Totals	22	89	117	1	212	1	257	0	172	0	184	2	138	1	190	1	138	1
Total day- light hours above 60°F. ^d	---	---	---	---	---	---	---	---	62.3	73.5	69.8	47.2	75.4	81.4				

a/ Actual release dates are as follows: 10/23/56; 10/28/56; 11/4/56; 11/8/56 and 11/13/56.

b/ During the 2-week period prior to release 4 of the 419 masses collected failed to hatch.

c/ Access to pen 9 was lost 12/8-11/1956 due to removal of bridge; pen was re-established 1 mile north of old location 12/12/56. Data for period 12/8-11/1956 is interpolated.

d/ Weather data is from Immokalee station No. 2, located approximately equidistant from the centers of the 3 areas.

Table 4.--Types of food provided, and mortality on indicated days, of screw-worm flies held in small cages (as used in mating tests) containing approximately 100 flies each.

Cage No's. :	Food ^{1/}	:Total number of flies dead :on indicated days (food :removed on 3rd day).		
		:3rd day	:4th day	:5th day
1-4	Honey-meat	7	227	397
5-8	Honey-meat + H ₂ O (Std.)	7	74	349
9-12	Honey	7	18	219
13-16	50-50 Honey-H ₂ O	3	8	153
17-20	Honey + H ₂ O	1	11	184
21-24	H ₂ O	350	418	---
25-28	2 Sugar lumps	87	323	406
29-32	2 Sugar lumps + H ₂ O	2	3	153
33-36	No food nor H ₂ O	381	420	--

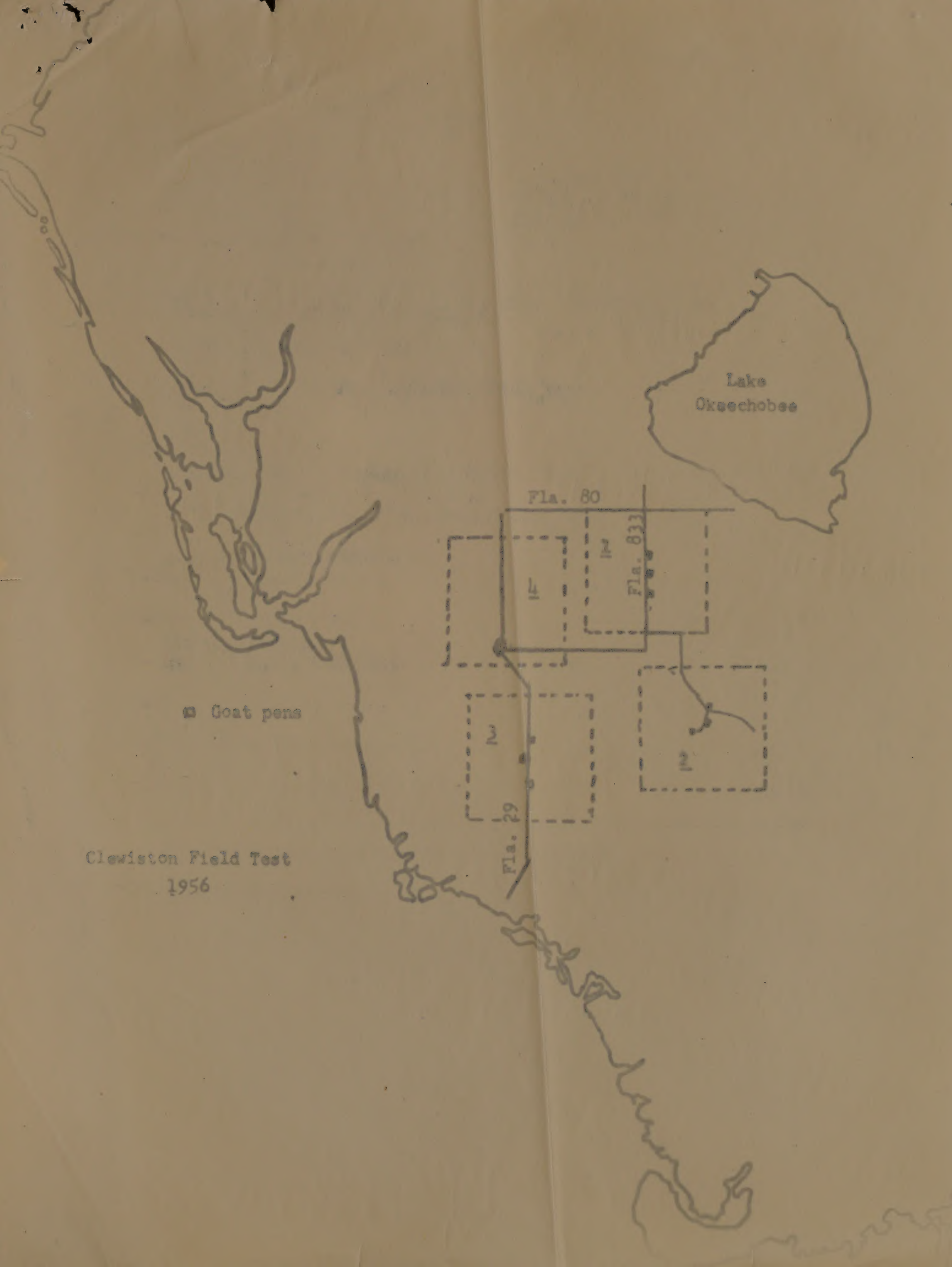
^{1/} Indicates materials mixed together; + indicates materials in separate containers.

Table 1.---Types of food provided, and mortality on indicated days, of screw-worm flies held in small cages (as used in making tests) containing approximately 100 flies each.

Cage No's.:	Food:	3rd day: 4th day: 5th day:	Removed on 3rd day:	on indicated days (food):	Total number of flies dead:
1-4	Honey-meat	7	527	327	
5-8	Honey-meat + H ₂ O (3rd.)	7	74	319	
9-12	Honey	7	18	319	
13-16	50-50 Honey-H ₂ O	3	8	153	
17-20	Honey + H ₂ O	1	11	184	
21-24	H ₂ O	350	118	--	
25-28	2 Sugar Jumps	87	323	406	
29-32	2 Sugar Jumps + H ₂ O	2	3	153	
33-36	No food nor H ₂ O	381	150	--	

Indicates materials mixed together; + indicates materials

in separate containers.



Lake
Oksechobee

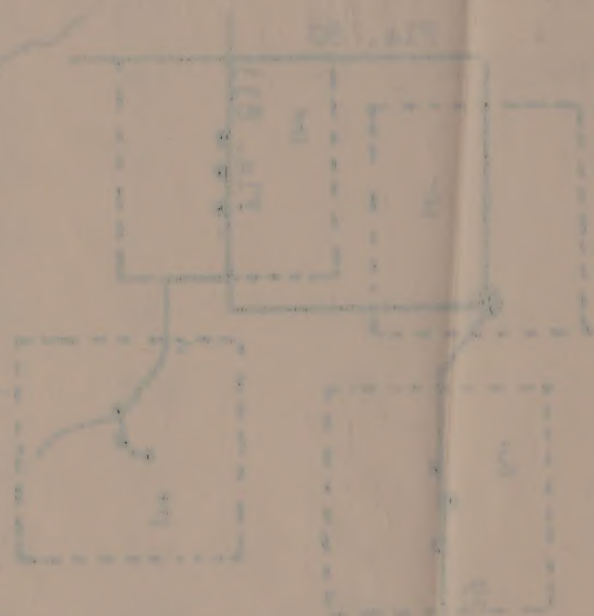
Fla. 80

Fla. 833

Goat pens

Clewiston Field Test
1956

Fla. 29



at Coast Line

Cleveland Field Test
1950